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Cloning and characterization of a novel human olfactory UDP-glucuronosyltransferase

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Xenobiotic metabolizing enzymes in the olfactory epithelium have been suggested to catalyse inactivation and facilitate elimination of odorants. We report here the molecular cloning and functional characterization of a human olfactory UDP-glucuronosyltransferase (UGT). The cloned protein is composed of 527 amino acids with an identity of 87% with a rat olfactory UGT and of 43–62% with other human UGT isoforms. Based on the sequence homology, it has been designated hUGT2A1. The gene was mapped to chromosome 4q13 by fluorescence *in situ* hybridization. The expression appeared to be specific for the olfactory tissue. The substrate specificity of this isoform was

assessed using Chinese hamster V79 cells stably transfected with the isolated cDNA. The expressed enzyme showed a broad substrate spectrum including a range of phenolic compounds as well as aliphatic and monoterpenoid alcohols, among them many odorants. Furthermore, some steroids, especially androgens, some drugs and carcinogens were conjugated. The results support a role of the enzyme in olfactory perception and in protection of the neural system against airborne hazardous chemicals.

Key words: airborne chemicals, detoxification, glucuronidation, olfactory enzymes.

INTRODUCTION

Conjugation with glucuronate is a major metabolic pathway to inactivate and eliminate endogenous as well as xenobiotic substances or, occasionally, to produce more biologically active compounds [1–3]. Based on their sequence similarities, UDP-glucuronosyltransferases (UGTs) have been grouped into two families, UGT1 and UGT2 [4]. The human UGT1 isoforms are derived from a single gene locus [5,6] and include enzymes that conjugate bilirubin, phenols, amines and carboxylic acids [3]. The human UGT2B enzymes, encoded by separate but similar genes, are particularly involved in steroid conjugation [7–11]. UGTs are expressed predominantly in liver, but extrahepatic expression, as in kidney [12,13], intestine [12,14,15] and brain [16], has been increasingly recognized.

Detoxifying enzymes are particularly important in epithelia exposed to xenobiotic chemicals. The olfactory neuroepithelium, which lines the posterior nasal cavity, is such an epithelium, exposed to a wide range of odorants as well as airborne toxic compounds. Odorants, which are mostly small lipophilic molecules, enter the mucus flow and reach the odorant receptors on sensory neurons. Odorant sensing is generally a transient process, requiring an effective signal termination, which could be provided by biotransformation of the odorant in the epithelial supporting cells [17]. Cytochrome P450-dependent mono-oxygenase activity is high in olfactory tissue and olfactory-specific isoforms have been identified and cloned [18,19]. Lazard et al. identified a major protein in bovine olfactory epithelium as a UGT, and subsequently cloned a rat olfactory specific UGT [20,21], which has been classified in its own subfamily, Ugt2A [4]. They further demonstrated that glucuronidation of a number of odorants abolished their ability to stimulate the receptors [21]. For a male

steroid pheromone in fish, however, it was shown that glucuronidation creates an extremely potent odorant [22,23]. Thus glucuronidation could be involved in both the termination and initiation of olfactory stimuli.

In the present study we report the molecular cloning of a novel human UGT, specifically expressed in the olfactory epithelium. The substrate spectrum and specificity of the enzyme was characterized using mammalian cells stably transfected with the isolated cDNA and a wide range of possible aglycone substrates, providing an indication of its possible functions in human odorant sensing and in detoxification of airborne toxic compounds.

EXPERIMENTAL

Materials

[α -³²P]dCTP (111 TBq/mmol) and UDP-[U-¹⁴C]glucuronic acid (10 GBq/mmol) were obtained from DuPont NEN (Boston, MA, U.S.A.). *Taq* DNA polymerase and restriction enzymes were from Promega (Southampton, Hants, U.K.), tissue-culture media and supplements, including G418 (Geneticin), were from Gibco-BRL Life Technologies (Paisley, Scotland, U.K.). A human brain 5'-STRETCH PLUS cDNA library, prepared from whole cerebral brain of a caucasian male, was obtained from Clontech (Palo Alto, CA, U.S.A.). Aglycone substrates for glucuronidation assays were obtained from Sigma-Aldrich (Poole, Dorset, U.K.).

cDNA cloning of a human olfactory UGT

A human olfactory cDNA library was kindly provided by Professor D. Lancet, Weizmann Institute of Science, Israel. The

Abbreviations used: RT, reverse transcriptase; UGT, UDP-glucuronosyltransferase.

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The nucleotide sequence data reported in this paper will appear in the EMBL and GenBank nucleotide sequence databases under the accession number AJ006054.

library contained cDNA from the olfactory epithelium tissue, surgically removed from a female patient, cloned into Uni-ZAP[®]XR Lambda vector (Stratagene). PCR primers chosen from the 3' half of the rat olfactory UGT cDNA sequence [21] were used to obtain a specific human sequence from this library. The sequences of the forward and reverse primers used were 5'-GAGACTATGGGGAAAGCTG-3' and 5'-CATGACAACTCAATCCAG-3' (bases 809–827 and 1453–1471, rat sequence), respectively. PCR was run at 94 °C for 1 min, at 52 °C for 1 min and at 72 °C for 2 min for a total of 35 cycles using *Taq* DNA polymerase. The obtained major PCR product was subcloned into the pGEM[®]-T Easy vector (Promega), subjected to DNA sequencing, subsequently labelled with [α -³²P]dCTP using the random-primer procedure [24], and used as a probe for screening the library by plaque hybridization. The phages were grown in XLI-Blue host cells and transferred from plates on to nitrocellulose filters (Hybond N⁺, Amersham). Hybridization of the filters was performed at 65 °C in hybridization buffer containing 6 × SSC (where 1 × SSC is 150 mM NaCl/15 mM sodium citrate, pH 7.0), 5 × Denhardt's reagent, 0.1 % SDS and the ³²P-labelled cDNA probe for 12 h followed by three high-stringency washing steps (2 × SSC/0.5 % SDS; 0.5 × SSC/0.1 % SDS; and 0.25 × SSC/0.1 % SDS; each at 65 °C for 30 min). Positive clones were then plaque-purified by secondary and tertiary screening and the pBluescript phagemid, containing the cDNA insert, was obtained by *in vivo* excision procedure. Several partial clones were obtained, and fragments of these were used for further rounds of screening, yielding a full-length clone of approximately 2.8 kb.

DNA sequencing

DNA sequence analysis was performed in both directions using double-stranded cDNA as a template. An ABI 377 sequencer (Perkin-Elmer-ABI, Foster City, CA, U.S.A.) was used in combination with a kit (Big Dye Terminator Cycle Sequencing Kit, ABI).

Fluorescence *in situ* hybridization

A 0.9-kb fragment of the 5' half of the human olfactory UGT cDNA was used for probe preparation. The fragment was obtained by PCR using the following primers: 5'-CTGCATC-AAGCCACATCATG-3' (bases 47–66) and 5'-ATCCCAGAG-AAAACACCACA-3' (bases 969–988). The cDNA fragment was nick-translated with SpectrumRed[®]-labelled dUTP using the Vysis Nick Translation Kit (Vysis, Downers Grove, IL, U.S.A.) according to manufacturer's protocol, except that salmon sperm DNA was used as carrier instead of human placental DNA. The labelled probe was hybridized to denatured human lymphocyte chromosome spreads overnight at 37 °C. The slides were washed with 0.4 × SSC/0.3 % Nonidet P40 and 1 × SSC/0.1 % Nonidet P40 each for 2 min at 73 °C, and with 2 × SSC/0.1 % Nonidet P40 for 1 min at room temperature. Chromosomes were counterstained with DAPI (4',6-diamidino-2-phenylindole hydrochloride) antifade (Vysis) and analysed under an Olympus BX 60 fluorescence microscope fitted with a Sensys cooled charge-coupled device camera and using the Vysis Quips[®] image-analysis software.

Stable expression in mammalian cells

Chinese hamster lung fibroblasts V79 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10 % (v/v) fetal calf serum and 100 units/ml penicillin/streptomycin. The complete cDNA insert of the isolated full-length clone was

excised from the pBluescript phagemid, and cloned into the *Bam*HI/*Xho*I site of the mammalian expression vector pcDNA3/Neo (Invitrogen, Groningen, The Netherlands). The correct orientation and integrity of the cDNA in the expression vector was assessed by restriction analysis and DNA sequencing of the cloning site. V79 cells were transfected with the pcDNA3/Neo cDNA construct or the vector only using Lipofectin reagent (Gibco-BRL) according to the manufacturer's instructions. After 48 h the cells were split and stable transfectants were selected using medium containing 1 mg/ml G418. Resistant clones were screened by reverse transcriptase (RT)-PCR, immunoblotting and functional assay for UGT expression. Sodium butyrate (2 mM) was added to the cells 24 h before harvesting to enhance the expression of the recombinant protein [25,26].

RNA isolation, reverse transcription and PCR

Total RNA was isolated using the RNAeasy Kit (Qiagen, Crawley, West Sussex, U.K.) according to manufacturer's instructions and DNase I-treated to prevent DNA contamination. The first strand of cDNA was synthesized from 2 µg of total RNA using M-MLV RT (Promega) and oligo random primers (250 ng). To screen for expression of the new UGT isoform, PCRs from reverse-transcription mixtures or cDNA libraries were performed with the forward and reverse primers 5'-CTG-CATCAAGCCACATCATG-3' (bases 47–66) and 5'-TCCCAT-GATTCCAAAGAGT-3' (bases 736–755), respectively, amplifying a 709-bp fragment specific for the human olfactory UGT. PCR was run at 94 °C for 1 min, at 55 °C for 1 min, and at 72 °C for 2 min for 30 cycles. As an internal control, a primer pair amplifying a 285-bp fragment from human β -actin (Promega) were used.

Northern-blot analysis

Total RNA (30 µg) were fractionated on a 1.2 % formaldehyde/agarose gel and transferred on to nitrocellulose membranes (Hybond N⁺, Amersham) with 20 × SSC buffer. The blot was then pre-incubated in hybridization buffer for 4 h at 65 °C, and hybridized for 12 h at 65 °C with the ³²P-labelled 709-bp fragment, obtained by PCR with the human olfactory UGT-specific primers (see above), with a β -actin control probe, or a probe directed against the 3' half of *hUGT2B4* (GenBank/EMBL accession number Y00317). A pre-made mRNA blot (Northern Territory[®] mRNA REAL[®] Human Normal Blot 1, Invitrogen) was hybridized under identical conditions. Membranes were washed with high stringency (2 × SSC/0.2 % SDS; 0.5 × SSC/0.1 % SDS; and 0.1 × SSC/0.1 % SDS; each at 65 °C for 20 min). Autoradiography was performed at –70 °C for 1–4 days.

Immunoblot analysis

Microsomes (100 000 g pellets; 50 µg of protein) prepared from cell homogenates were separated on an SDS/polyacrylamide gel (9 % gel). Immunoblotting was performed essentially according to Towbin et al. [27] with a tank blotting system and an enhanced chemiluminescence horseradish peroxidase detection system (DuPont NEN). The anti-rat liver UGT antiserum (RAL) was raised in sheep as described [28,29].

Glucuronidation assay

cDNA-transfected and vector-transfected (control) V79 cells were resuspended in PBS and disrupted by sonication (four times for 5 s). UGT activities towards various substrates were analysed as described [30] with the following modifications. Enzyme-assay

mixtures (50 μ l final volume) contained 100 mM Tris/maleate (pH 7.4), 10 mM $MgCl_2$, 0.5 mM UDP-[^{14}C]glucuronate (7.4 kBq), 100 μ g of homogenate protein and the aglycone substrate at concentrations indicated, added from a stock solution in an appropriate solvent (for most compounds, DMSO). In the initial screening for substrates, incubations with 50 μ M UDP-[^{14}C]glucuronate (7.4 kBq) were performed. 5'-AMP (4 mM) was added to inhibit the degradation of UDP-glucuronate by nucleotide pyrophosphatase. Incubations were terminated by addition of ethanol, and precipitated proteins were removed by centrifugation. The supernatants were subjected to HPLC analysis with radioactivity detection, using a C_{18} Spherisorb column and an ammonium acetate/acetonitrile gradient [30]. Apparent K_m values were determined at protein concentrations and reaction times yielding linear product formation and substrate concentrations varying from 0.01 to 1 mM. The K_m value for UDP-glucuronate was determined using variable concentrations (0.05–1 mM) of the UDP-sugar at fixed concentrations of 3-hydroxybiphenyl or citronellol (1 mM).

RESULTS

Cloning and sequence analysis of the human olfactory UGT cDNA

In order to isolate a human olfactory UGT cDNA, we used PCR primers based on the rat olfactory UGT cDNA sequence (GenBank/EMBL accession number X57565) in two highly conserved regions, as was indicated by sequence homology with human UGT2B isoforms. With human olfactory cDNA as a template, PCR with these primers resulted in the amplification of a major 0.6-kb product (results not shown). Sequence analysis of this fragment revealed a high degree of homology with the rat olfactory sequence. Subsequently, several clones with overlapping sequences were obtained by screening the library with this fragment, with one containing the largest insert of approx. 2.8 kb. The cDNA sequence (Figure 1) revealed a first in-frame ATG codon located at nucleotides 64–67, and a stop codon at positions 1645–1647, followed by 1.1-kb non-coding sequence ending in a polyadenylation signal and a poly-A stretch (results not shown). The sequence context around the translation start codon fulfilled the Kozak consensus [31]. The 1581-bp open reading frame encoded a protein of 527 amino acids, which is in agreement with the length of other UGT proteins (Table 1). Sequence comparison with the rat olfactory UGT revealed 85 and 87% identities at the cDNA and deduced amino acid levels, respectively. The similarity of the protein sequences was 91%. The overall identity with other human UGTs was about 45% for members of the UGT1A subfamily and about 60% for human UGT2B isoforms in a gapped alignment (Table 1). The N-terminal half of the protein, which is thought to contain the aglycone binding site [3], however, exhibited a significantly lower degree of identity (from 24% for hUGT1A9 to 40% for hUGT2B4) than the C-terminal half (57% for UGT1A and about 80% for UGT2B family members). The identity with the rat Ugt2a1 was above 80% in the C-terminal as well as in the N-terminal domain (Table 1). The high sequence similarity with the rat olfactory sequence suggests the name hUGT2A1. Figure 2 illustrates a cluster analysis of cloned human UGTs with the olfactory UGT as the first human member of the UGT2A branch.

Chromosomal localization of the hUGT2A1 gene

The localization of the respective gene was determined by means of fluorescence chromosomal *in situ* hybridization. To minimize cross-hybridization of conserved parts of the cDNA to other

AAACTTCAACAGAGAAAAATTTCCTTCACATCTCATCAATCTCTGCATCAAGCCACATC		
64	ATGTTAAACAACTTCCTGCTGTTCTCCCTTCAGATAAGCTCATAGAACCACTCTTGTT	123
1	I M L N N L L L F S L O I S L I G T T L G	20
124	GGGAATGTTTGGATTTGGCCAAATGGAAGGTAGCTATGGCTAAATGTTAAGATAATTATA	183
21	G N V L I W P H E G S H W L N V K I I I	40
184	GATGAGCTCATTAAGGAGCATAATGTGACTGCTAGTTCCTGCTGCTGCTGCTTTC	243
41	D E L I R K E H N V T V L V A S G A L F	60
244	ATCACCAACCTCTAACCCATCTCTGACATTTGAAATATATAAGGTGCTTTCGGCAA	303
61	I T P T S N P S L T F E I Y K V P F G K	80
304	GAAAGATAGAAGGATTAATTAAGGACTTCGTTTCGACATGGCTGGAATAGACCATCT	363
81	E R I E G V I R D F V S T W L E N R P S	100
364	CCTTCAACATTTGGAGATTCTATCAGGAGATGGCCAAAGTAATCAAGGACTTCCACATG	423
101	P S T I W R F Y Q E M A K V I K D H F M	120
424	GTGCTCAGGAGATCTGTGATGGCTTCTTAAACCAACAGCTGATGGCAAGCTAAG	483
121	V S Q E I C D G V L K N Q O L M A K L K	140
484	AAAAGCAAGTTGAAGTCTGGTGTCTGATCCAGTATTTCTTGTGGGATATAGTAGCT	543
141	K S K F E V L V S D P V F P C G D I V A	160
544	TTAAACTTGAATTCCTATTATGTACTCTTGGAGTTTCTCCAGCTCAACAGTGGAA	603
161	L K L G I P F M Y S L R F S P A S T V E	180
604	AAGCACTGTGGGAAGGTACCATACCTCTCTCTATGCTCTGCTGTTTATCAGAACTC	663
181	X H C G K V P Y P P S Y P A V L S E L	200
664	ACCGACCAATGCTTCTCACTGACAGAATAAGAAATTTATCTCTACCACTACAGGAC	723
201	T D Q M S P T D R I R N F I S Y H L Q D	220
724	TACATGTTGAACTCTTTGGAATCATGGGATTCATATACTAGTAAGCTTTAGGAAGA	783
221	Y M F E T L W K S W D S Y Y S K A L G R	240
784	CCCACTACGTTATGTGAGACTATGGGAAAGCTGAAATTTGGTTAATCCGAACATA1TGG	843
241	P T T L C E T M G K A E I W L I R T Y W	260
844	GATTTGAAATTCCTGCTCATACTTACCTAAATTTGAGTTTGTGGAGGATTCGACTGC	903
261	D F E F P R P Y L P N F Y E F V G G L H C	280
904	AAACCTGCCAACTTTACCTAAGGAAATGGAAGATTTATCCAGAGCTCAGTAAAAAT	963
281	K P A K P L P K E M E E F I O S S G K N	300
964	GGTGTGTGTGTTTCTCTGGGATCAATGGTCAAAACCTTACAGAAGAAAGGCCAAT	1023
301	G V V V F S L G S H V K N L T E E K A H	320
1024	CTTATGCTCAGCCCTTCCAGATTCACAGAGGTTTATGGGATACAAAGGAAG	1083
321	L I A S A L A O I P Q K V L R Y K G K	340
1084	AAACAGCCACATTAGGAACAATACTCAGCTCTTGTGATGGATACCCAGAAATGATCTT	1143
341	K P A T L G N N T O L F D W I P Q N D L	360
1144	CTTGACATCCCAAAACCAAGCTTTATCACTCATGGTGGAACTAATGGGATCTACGAA	1203
361	L G H P K T K A F I T H G G T N G I Y E	380
1204	GCTATTACACGGAGTCCCTATGGTGGGAGTCCCATGTTTGTGATCAGCCTGATAAC	1263
381	A I Y H G V P H V G V P H F A D Q P D N	400
1264	ATTGCTCACATGAAGCCAAAGGAGCAGCTGTGGAGTGAACTTAAACACAATGACAAGT	1323
401	I A H N K A K G A A V E V N L N T M T S	420
1324	GTGGATTGCTTAGCGCTTGTGAGACAGTCATTATGAACCTTCTTATAAGAGAAATGCT	1383
421	V D L L S A L R T V I N E P S Y K E H A	440
1384	ATGAGTTATCAAGAAATCACCATGATCAACCTGTAAGGCCCTGGATGAGCAGCTTTC	1443
441	M R L S R I H H D Q P V K P L D R A V F	460
1444	TGGATCGAGTTTGTATGCGCCACAAAGGAGCCACCTCGGTTGACAGCCATGAC	1503
461	W I E F V M R H R G A K H L R V A A H D	480
1504	CTCACCTGTTCCAGTACCACTCTTTGGATGTAATGGGTTCTTGTGCTGTGTGACA	1563
481	L T W F Q Y H S L D V I G F L L V C V T	500
1564	ACGGCTATATTTTGGTCATCAATGTTGTTTGTCTGTCATCAAAATTTGGTAAGATA	1623
501	T A I F L V I Q C C L F S C Q K F G K I	520
1624	GGAAAGAGAAAAAAGAGAAATAG	
521	G K K K R E *	
GTCAAGAAAAAGAGAAATATATATCTTAAGTTTGGCAAAATCCTGAGTAGTGGAGTCTATTAATTCAGAC		
AAAAGGGAGTTTACAAGAACAGCTCTTCATCTCGTTTCCAAA		

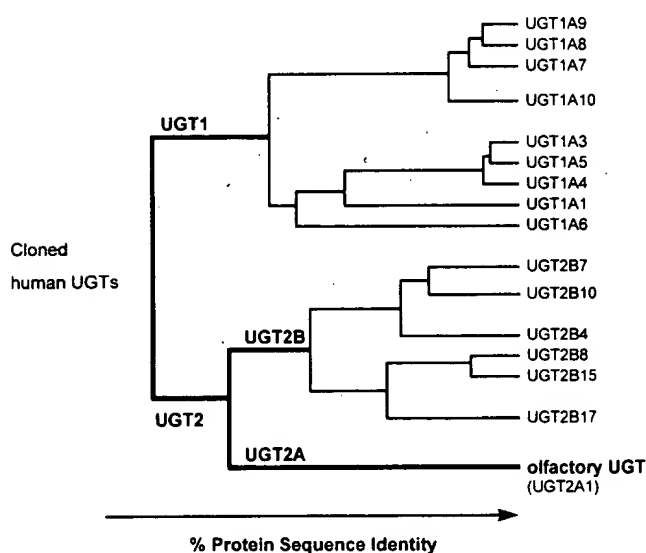
Figure 1 Nucleotide and deduced amino acid sequences of the human olfactory UGT

The nucleotide sequence of the isolated clone contains a 1581-bp open reading frame encoding 527 amino acids. From the 1.1-kb non-coding 3' sequence only 121 bp are given. The stop codon is indicated by an asterisk.

Table 1 Sequence comparison of amino acids in the human olfactory UGT (*hUGT2A1*) with the rat olfactory *Ugt2a1* and with other members of the human UGT family

Data were obtained from alignments generated by the GAP program of the sequence-analysis software package of the University of Wisconsin Genetics Computer Group. In addition to the amino acid identity over the entire sequence (overall), the percentage of identities in the N-terminal (amino acids 1–240) and C-terminal (amino acids 241–527) domains are given. acc. no., accession number.

Name	GenBank/EMBL acc. no.	Amino acids	hUGT2A1 identity (%)			Overall similarity (%)
			N-terminal	C-terminal	Overall	
hUGT2A1	—	527	100.0	100.0	100.0	100.0
rUgt2a1	X57565	527	84.2	89.9	87.3	90.9
hUGT1A1	M57899	533	30.4	57.1	45.6	55.0
hUGT1A9	S55985	530	24.2	57.8	43.2	52.0
hUGT2B4	Y00317	528	40.0	78.7	61.6	70.2
hUGT2B7	J05428	529	38.3	79.8	61.1	70.4
hUGT2B8	U06641	527	35.4	78.1	59.2	69.3
hUGT2B10	X63359	528	34.2	78.1	58.4	67.6
hUGT2B15	U08854	523	35.4	79.1	59.8	70.0
hUGT2B17	U59209	530	37.5	80.1	61.0	70.7

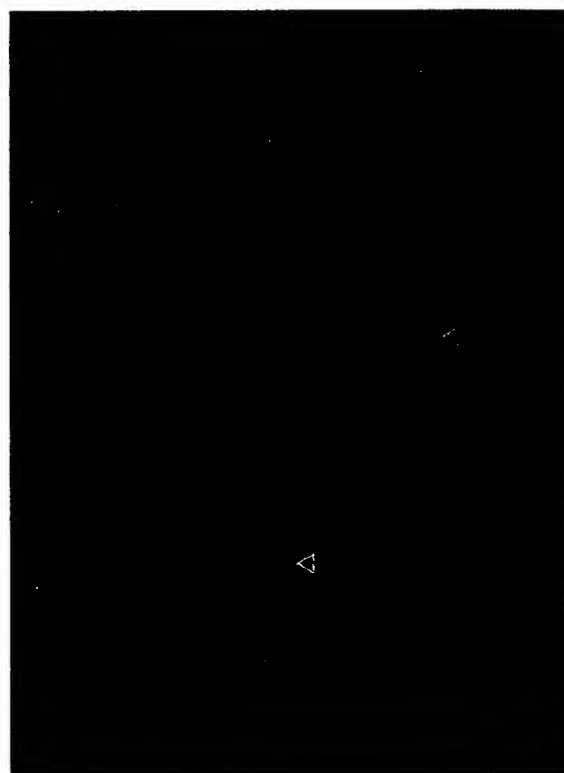
**Figure 2** Cluster analysis of the protein sequence identity of cloned human UGTs, including the olfactory UGT

The horizontal branch lengths are inversely proportional to the degree of identity between the amino acid sequences. All sequences, except for the human olfactory, were taken from the GenBank/EMBL databases. For accession numbers see Table 1 and [4]. The UGT Nomenclature Committee suggested the name human UGT2A1 for the new isoform.

UGT genes, a fragment of the 5' half, probably derived only from the first exon and showing a low degree of identity (< 30%) with other UGTs, was used as a probe. A fragment from the same region was used in Northern-blot analysis and was shown to be isoform-specific (see below). With this probe a specific fluorescent signal was observed on both chromosome 4 homologues at band q13 on both chromatids (Figure 3).

Tissue-specific expression of *hUGT2A1*

As a first step towards characterizing the tissue-specific expression, we screened through cDNA libraries from other human tissues by PCR with primers amplifying a 709-bp product (Figure

**Figure 3** Chromosomal localization of the human olfactory UGT (*hUGT2A1*) gene

Fluorescence *in situ* hybridization on human metaphase spreads was performed using a *hUGT2A1*-specific probe. Fluorescence can be observed on chromosome 4q13 (indicated by arrowheads).

4A). This specific fragment was used, in addition, as a probe in Northern-blot analysis (Figure 4B). Hybridization of this probe was observed with RNA from *hUGT2A1*-transfected V79 cells (see below), but not with RNA from cells expressing other UGT2 isoforms (Figure 4B, upper left panel). With this probe, no transcript could be detected on the Northern blot with mRNA

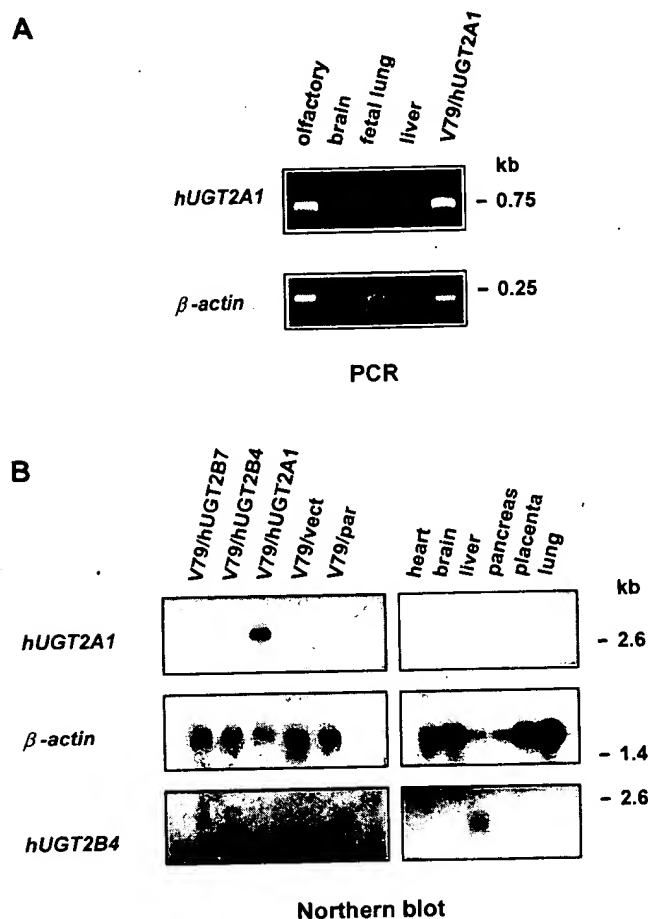


Figure 4 Distribution of human olfactory UGT (hUGT2A1) mRNA

Tissue-specific expression was investigated by PCR in human cDNA libraries (**A**) and by Northern blot analysis (**B**). (**A**) Human cDNA libraries prepared from olfactory epithelium, total brain, fetal lung and liver were screened with PCR primers amplifying an olfactory UGT-specific fragment (hUGT2A1), and with β -actin primers as control. In addition, RNA prepared from olfactory UGT-transfected V79 cells (V79/hUGT2A1) was subjected to RT-PCR with the same primer pairs. (**B**) Total RNA (20 μ g) from parental (V79/par), vector-transfected (V79/vect), and hUGT2A1-, hUGT2B4- and hUGT2B7-transfected V79 cells separated on a 1.2% formaldehyde/agarose gel (left blot) and mRNA (2 μ g) from the indicated human tissues (right blot) were hybridized with a 32 P-labelled probe specific for the olfactory sequence (hUGT2A1), β -actin or hUGT2B4.

from human heart, total brain, liver, pancreas, placenta or lung (Figure 4B, upper right panel). A β -actin probe served as control for integrity of the RNA (Figure 4B, middle right panel: note that β -actin expression levels vary depending on the tissue type). As a further control, a probe directed against the 3' half of human UGT2B4 was used. This probe detected hUGT2B4 and hUGT2B7 mRNAs (Figure 4B, lower left panel) and gave an intensive signal in liver on the multiple tissue blot (Figure 4B, lower right panel).

In the more sensitive PCR, no hUGT2A1 expression was found again in liver. However, there was a significant band from the human brain library in addition to the olfactory epithelium (Figure 4A).

Heterologous expression of hUGT2A1 in V79 cells

To characterize the enzyme function and substrate specificity the entire cDNA insert of the isolated clone was cloned behind the

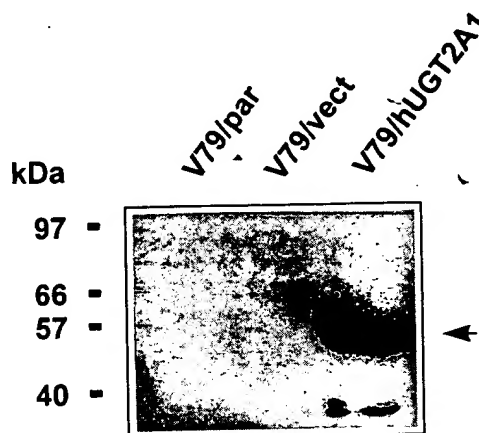


Figure 5 Immunoblot analysis of the human olfactory UGT (hUGT2A1) expressed in V79 cells

Microsomes (100 000 g sediments) prepared from parental (V79/par) and vector-transfected (V79/vect) control V79 cells, and from cells transfected with the human olfactory UGT cDNA (V79/hUGT2A1) were separated by SDS/PAGE and transferred on to a nitrocellulose membrane. Equal amounts (50 μ g) of protein were loaded. The membrane was immunostained using an anti-rat liver UGT antiserum (RAL), which binds to epitopes in the conserved C-terminal domain of both UGT1 and UGT2 family members.

cytomegalovirus promoter of a mammalian expression vector and transfected into Chinese hamster V79 cells. The expression in stable transfectants was examined at the mRNA level by RT-PCR and Northern blotting (Figures 4A and 4B, left panel) and at the protein level in an immunoblot with an anti-UGT antiserum (Figure 5). A transcript of approx. 2.8 kb in the Northern-blot analysis and a protein of about 55 kDa was detected only in the cDNA-transfected and not in the parental or vector-transfected control cells.

Substrate specificity of hUGT2A1

The UGT activity of the enzyme was assessed using cell homogenates from this stably hUGT2A1-expressing cell line, with a variety of aglycone substrates and 14 C-labelled UDP-glucuronate. Conjugates formed were separated and quantified by HPLC. UGT activity was not detected with homogenates from vector-transfected control V79 cells for the substrates used in this study. hUGT2A1-catalysed glucuronide formation was observed for simple planar phenols, including odorants like vanillin and eugenol (Table 2). However, a bulky substituent in the *ortho* position to the hydroxy group seemed to hinder the glucuronide formation since thymol and carvacrol, with an *ortho*-isopropyl and methyl group respectively, were not converted by the enzyme under the same conditions. In addition, 3- and 4-hydroxybiphenyl showed the highest conversion rates amongst the phenolic compounds tested, whereas glucuronide formation with 2-hydroxybiphenyl was low. The transferase also catalysed the glucuronidation of coumarins, particularly 7-hydroxylated derivatives such as umbelliferone and scopoletin, at high rates. The flavonoids 3- and 7-hydroxyflavone as well as the anthraquinone anthraflavic acid were also converted.

Furthermore, aliphatic alcohols, of which many are perceived as odorants, were good substrates for hUGT2A1, including both stereoisomers of β -citronellol, ethylhexanol, geraniol and cinnamyl alcohol. In addition, some monoterpenoid odorants like borneol were substrates. Menthol was converted, however, with a relatively low rate and a preference for the (+) isomer.

Table 2 Substrate specificity of the human olfactory UGT expressed in V79 cells

Substrates (1 mM) were incubated with homogenates of *hUGT2A1*-transfected V79 cells (100 µg of protein) in the presence of UDP-[¹⁴C]glucuronate (0.5 mM) for 1 h at 37 °C. Glucuronide formation was determined by HPLC analysis as described in the Experimental section. Mean values are from triplicate determinations; S.D. values were within 20% of the means.

Aglycone	UGT activity (nmol/mg of protein per h)
Phenolic compounds	
Vanillin*	44.1
4-Hydroxyanisole*	31.2
Eugenol*	19.2
Thymol*	< 0.1†
Carvacrol*	< 0.1†
3-Hydroxybiphenyl	146.4
4-Hydroxybiphenyl	108.1
2-Hydroxybiphenyl	7.8
1-Naphthol	45.4
Octyl gallate	13.0
Coumarins	
Scopoletin*	53.9
4-Methylumbelliferone	40.8
Umbelliferone*	36.0
4-Hydroxycoumarin	0.5
Flavonoids	
3-Hydroxyflavone	19.2
7-Hydroxyflavone	9.8
Anthraflavic acid	12.6
Drugs	
Morphine‡	4.4
Ibuprofen*	4.1
Valproic acid*	1.9
<i>N</i> -(2-Hydroxyethyl)aniline	5.1
Aliphatic compounds	
<i>S</i> -(−)-β-Citronellol*	77.4
<i>R</i> -(+)-β-Citronellol*	60.6
2-Ethylhexanol	52.4
Geraniol*	20.2
Cinnamyl alcohol*	10.8
Linalool*	2.1
Monoterpenoid compounds	
(−)-Borneol*	41.1
(+)-Isomenthol*	5.8
(+)-Menthol*	4.8
(−)-Menthol*	1.9
Fenchyl alcohol*	< 0.1†
Steroids	
Testosterone	12.4
5α-Androstane-17β-ol-3-one	10.9
5α-Androstane-3α-17β-diol	6.3
β-Pregnane-3α-17α-diol-20-one	3.2
4-Hydroxyestrone	2.4
Estradiol	0.9
Estrone	< 0.1†
Estriol	< 0.1†
β-Pregnane-3α-20α-diol	< 0.1†
Amine compounds	
α-Naphthylamine	1.3
2-Amino biphenyl	< 0.1†

* The systematic names of these compounds are: (−)-borneol, (1*S*)-endo-1,7,7-trimethylbicyclo-(2,2,1)heptan-2-ol; carvacrol, 5-isopropyl-2-methylphenol; cinnamyl alcohol, 3-phenyl-2-propen-1-ol; β-citronellol, 3,7-dimethyl-6-octen-1-ol; eugenol, 4-allyl-2-methoxyphenol; fenchyl alcohol, 1,3,3-trimethyl-2-norbornanol; geraniol, 3,7-dimethyl-2,6-octadien-1-ol; 4-hydroxyanisole, *p*-methoxyphenol; ibuprofen, α-methyl-4-(isobutyl)phenylacetate; linalool, 3,7-dimethyl-3-hydroxy-1,6-octadiene; menthol, 5-methyl-2-(1-methylethyl)cyclohexanol; scopoletin, 7-hydroxy-6-methoxy coumarin; thymol, 5-methyl-2-isopropylphenol; umbelliferone, 7-hydroxycoumarin; valproic acid, 2-propylpentanoic acid; vanillin, 3-methoxy-4-hydroxybenzaldehyde.

† Glucuronide formation was not detectable.

‡ Glucuronide formation at pH 8.0.

Table 3 Kinetic parameters for different substrates of the human olfactory UGT

UGT activities and apparent K_m and V_{max} values for five representative substrates were calculated as described in the Experimental section. Means ± S.D. ($n = 3$) are shown.

Substrate	K_m (µM)	V_{max} (nmol/mg of protein per min)	V_{max}/K_m (µl/mg of protein per min)
3-Hydroxybiphenyl	75.4 ± 11.9	2.60 ± 0.18	34
Umbelliferone	59.5 ± 5.9	0.61 ± 0.02	10
<i>S</i> -(−)-β-Citronellol	51.6 ± 6.1	1.98 ± 0.04	38
Borneol	170.1 ± 14.1	1.05 ± 0.03	6
Testosterone	56.2 ± 9.7	0.25 ± 0.04	4
UDP-glucuronate*	251, 274†		

* The apparent K_m value for UDP-glucuronate was determined using 3-hydroxybiphenyl and *S*-(−)-β-citronellol as substrates.

† Only two determinations made.

Since human UGT2B isoforms are especially involved in steroid conjugation, the ability of *hUGT2A1* to conjugate steroids was investigated. Interestingly, the enzyme showed activity, especially towards androgens (Table 2). The preferential position in these compounds seemed to be the 17β-hydroxy group. Testosterone (4-androsten-17β-ol-3-one) and dihydrotestosterone (5α-androstan-17β-ol-3-one) exhibited the highest rates amongst the steroids tested, while an additional hydroxy group in the 3α position led to a decreased glucuronidation rate. β-Pregnane-3α-17α-diol-20-one, but not β-pregnane-3α-20α-diol, was a substrate, indicating that a 17α-, but not a 3α- or 20α-, hydroxy group can also serve as a glucuronidation site with this enzyme.

The low but significant activities observed with ibuprofen, valproic acid and α-naphthylamine indicate that, at least in certain compounds, a carboxyl or amine moiety can be used by the enzyme as a conjugation site. In addition to α-naphthylamine, *N*-(2-hydroxyethyl)aniline represents another genotoxic compound conjugated by the enzyme. Glucuronidation of morphine could also be detected (Table 2).

In Table 3, kinetic parameters are given for selected aglycones that are representative of the different classes of chemical compounds. The highest glucuronidation efficiency was found for (−)-citronellol with an apparent K_m value of 52 µM, followed by 3-hydroxybiphenyl with a slightly higher K_m value. Despite the lower conversion rates obtained with testosterone, the K_m value calculated for this steroid was almost the same as for citronellol.

DISCUSSION

In this study we described the isolation and expression of a novel human UGT isoform. The protein sequence showed a high similarity (91%) to a rat olfactory UGT [21], which has been systematically termed rat Ugt2a1 [4]. The respective human gene was localized to chromosome 4q13 (Figure 3), the same region where the *UGT2B* family is clustered [32]. However, the sequence identity to human UGT2B isoforms is significantly lower than the identity between the members of this subfamily (Figure 2). The assignment of UGT orthologues, especially in the UGT2B family, is often difficult because of recent species-specific gene duplications and gene-conversion events [4]. However, only one member of the human UGT2A family has been identified so far,

and the high homology between the new human isoform and rUGT2a1 indicates that they are most likely orthologues. The UGT Nomenclature Committee [4] consequently suggested the systematic name hUGT2A1. Our cloning experiments so far have provided no indication for the presence of other different UGT2A isoforms in the olfactory tissue, but their existence cannot be ruled out.

The preliminary study of the tissue distribution of hUGT2A1 indicated a specific expression in the nasal tissue. Expression could not be found in liver, by either Northern-blot or PCR analysis (Figure 4). An expression in certain parts of the brain, indicated by a positive PCR signal from a human whole brain cDNA library (Figure 4A), has to be confirmed in more detailed studies. mRNA of rUGT2a1 was detected similarly only in rat olfactory tissue and not in liver, kidney, lung or intestine [21].

Transiently expressed rUGT2a1 has been shown to conjugate the phenolic compounds eugenol, methylumbelliferone and 4-hydroxybiphenyl with rates decreasing in this order [21]. The same compounds were glucuronidated by the human enzyme but with the reverse ranking of activities (Table 2), indicating that the spectrum of substrates may be similar but that significant species differences exist in the preference for different substrates. hUGT2A1 showed high affinity to aliphatic odorants, exemplified by β -citronellol, the substrate most efficiently glucuronidated amongst the compounds tested (Table 3). This and the tissue specificity points to a role in olfaction, providing a means of turning off the olfactory signal, so that new stimuli can be received, or occasionally generating a more active stimulus. Other candidates for enzymes involved in this process are olfactory specific P450-dependent mono-oxygenases [18–20], glutathione S-transferases [33] and a sulphotransferase [34], identified and cloned from rodent nasal tissue. Besides compounds perceived consciously as odorants, hUGT2A1 showed a relatively high affinity towards some steroids, such as testosterone (Table 3). Steroidal compounds perceived by special subfamilies of odorant receptors in an unconscious manner are pheromones [35,36]. These molecules are known to be secreted by animals and then detected by other members of the same species, where they regulate such basic functions as mating, the timing of the oestrous cycle and aggressiveness [37]. However, little is known about the chemical nature and possible role of pheromones in humans to date. The glucuronide of 5 β -pregnane-3 α ,17 α -diol has been demonstrated to be a highly potent pheromone in piscine species [22], suggesting that for these compounds glucuronidation could be involved in the formation of active stimuli.

The olfactory epithelium is also an important barrier between the neural system and the environment. This tissue is exposed to a wide range of airborne chemicals, and it has been shown that compounds can be transported along the olfactory axons into the central nervous system, circumventing the blood-brain barrier [38,39]. Entry of pathogenic agents through the olfactory system has been suggested to be involved in the pathogenesis of neurodegenerative diseases, and anosmia is one of the early changes in Alzheimer's disease [40]. The broad substrate specificity of hUGT2A1, including hydroxylated aromatic compounds as well as amines (Table 2), makes it an excellent candidate for a detoxification enzyme providing, together with other olfactory enzymes, protection against airborne xenobiotics.

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